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From α-helix to β-sheet – a reversible metal ion induced peptide secondary structure switch†

Kevin Pagel, Toni Vagt, Tibor Kohajda and Beate Koksch*

Freie Universität Berlin, Institut für Chemie – Organische Chemie, Takustrasse 3, 14195, Berlin, Germany. E-mail: koksch@chemie.fu-berlin.de; Fax: +49-30-838-55644; Tel: +49-30-838-55344

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Here we introduce a peptide model based on an α-helical coiled coil peptide, providing a simple system which can be used for a systematic study of the impact of different metal ions in different oxidation states on peptide secondary structure on a molecular level; histidine residues were incorporated into the heptad repeat to generate possible complexation sites for Cu^{2+} and Zn^{2+} ions.

The transformation process from α-helices to β-sheet structures appears to be one of the major factors in the genesis and evolution of a variety of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease, and several prion diseases.[1,2] Although the formation of amyloid fibrils from the proteins involved, such as amyloid β (Aβ) and $PrP^{c/sc}$, is thought to be the pathogenic parameter for these diseases, the direct precursors are partially unfolded fragments of proteins or preliminary formed β-sheets, respectively. Metallochemical reactions are considered to be a common denominator for neurodegenerative diseases, as the concentration of heavier metal ions in brain tissue is naturally high.[3] The possibility that Cu^{2+} and Zn^{2+} ions play a significant role in assembling Aβ deposits in the brain of AD patients has been reported by Bush *et al.*[3] The conformation of strain variants of PrP^{sc} have also been reported to depend upon Cu^{2+} and Zn^{2+} interactions.[4] Therefore, investigation of the dependency of the α-helix to β-sheet conformational switch[5] on environmental factors on a molecular level is one of the main challenges to the detailed understanding of the pathways from incubation to mortality.[6]

We have now developed a peptide model which can serve as a versatile tool for studying the impact of metal ions on the transformation between α-helical and β-sheet peptide and protein structures.

The design is based on an antiparallel double stranded α-helical coiled coil motif.[7] The formation of a coiled coil is mediated by 2 different recognition domains expressed by the characteristic 4–3 heptad repeat primary structure. Positions of the heptad repeat are commonly denoted $(a–b–c–d–e–f–g)_n$. The stability of the dimer results from the side-by-side packing of hydrophobic residues in positions a and d of the heptad repeat which forms the first recognition domain, as well as the inter-helical salt bridges formed by charged residues in positions e and g of the second recognition domain.[8,9] Amino acid residues in positions b, c, and f of the heptad repeat do not directly influence the stability of dimer formation, but have a subtle impact *via* cooperative interactions within the coiled coil.[10]

Peptide CC is designed to fold into a weak α-helical coiled coil. In addition to the characteristic heptad repeat pattern, both peptides feature hydrophobic valine residues within the solvent-exposed b, c, and f positions, and neutral serine in the e and g positions, to make the whole system more prone to formation of β-sheet structures under ambient solution conditions.[11] To study the influence of metal ions on the secondary structure of helical peptides, we introduced four histidine residues per helix as ligation sites for metal ions, to give the peptide CCM.[12] Histidine is known to bind metal ions due to its high electron donor characteristics of the π and τ-nitrogen atoms. Therefore, His residues were placed within the heptad repeat, such that in the case of β-sheet formation efficient complexation of metal ions can happen. In the case of CCM, however, metal ions can be expected to either trigger a conformational change[13,14] or at least nucleate a certain conformation.[15] The primary structure of the two *de novo* designed 26 amino acid peptides which are described here is shown in Fig. 1.

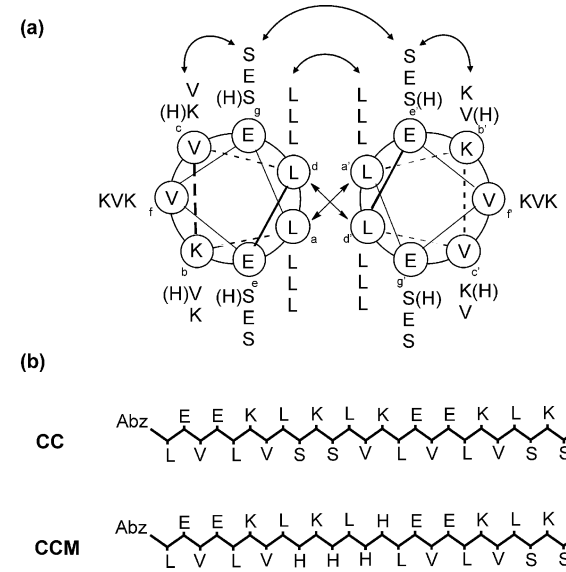

Fig. 1 Coiled coil based peptide (CC) and coiled coil based peptide for metal complexation (CCM). (a) Helical wheel model of CC and CCM (in parentheses), (b) schematic β-sheet layer; sequences shown correspond to the complete primary structure.

The influence of the differing environmental conditions on the secondary structure was followed by CD spectroscopy. As expected, both peptides show a typical β-sheet spectrum under aqueous conditions (10 mM phosphate buffer, pH = 7.4), which is characterized by a prominent minimum at 216 nm (Fig. 2).[16] The secondary structure at different peptide concentrations and pH values was investigated for both peptides. Within a concentration range of 25 to 100 μM, no noticeable difference in the β-sheet-forming tendency of the peptides CC and CCM was observed.[16] Different amounts of TFE were added in order to generate solvent conditions which are known to support formation of helical peptide folding.[17] The spectra acquired clearly show that under the influence of TFE the general propensity to switch from a β-sheet to an α-helical conformation (the latter indicated by two prominent bands at 208 and 222 nm) is equal for both peptides.

Due to the fact that both CC and CCM prefer a helical conformation at 40% TFE (Fig. 2),[16] these conditions were

† Electronic supplementary information (ESI) available: Experimental details. See http://www.rsc.org/suppdata/ob/b5/b505979h/

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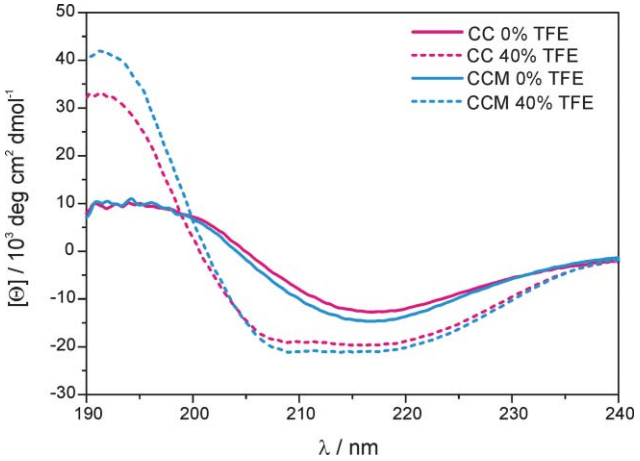

Fig. 2 CD spectra of peptide CC (—) and CCM (---) in buffer (10 mM Tris-HCl, pH 7.4; peptide concentration 0.1 mM) and at 40% TFE.

chosen for further investigations of the peptide secondary structure at different Cu^{2+} and Zn^{2+} ion concentrations.

As expected, addition of one equivalent of Cu^{2+}, as well as Zn^{2+}, to a 0.1 mM solution of peptide CC, which does not contain any histidine residues, does not result in any significant change of its secondary structure (data not shown). In contrast, the addition of Cu^{2+} and Zn^{2+} ions, respectively, has a strong impact on the folding of peptide CCM (Fig. 3), which shows a vigorous switch from the mainly α-helical conformation to a secondary structure that is mainly β-sheet.

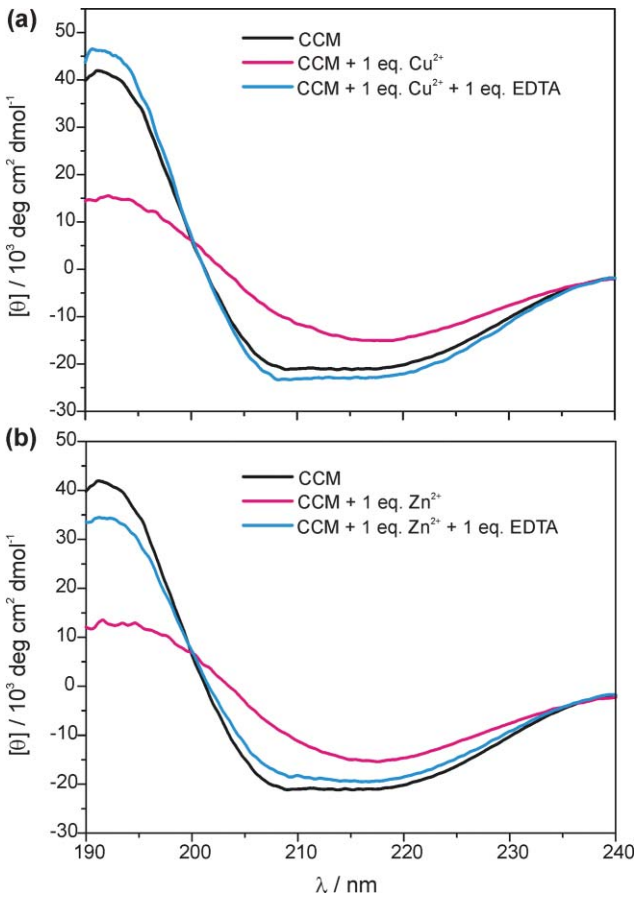

Fig. 3 CD spectra of peptide CCM in 40% TFE at pH 7.4 and at a peptide concentration of 0.1 mM with (a) 0.1 mM $CuCl_2$/EDTA and (b) 0.1 mM $Zn(OAc)_2$/EDTA.

The influence of the metal scavenger EDTA was investigated in order to analyze a potential reversibility of the secondary structure switch process. The addition of equimolar amounts of EDTA (Fig. 3) results in a complete recovery of the primarily helical structure. The secondary structure switch can thus be reversed by simply capturing the β-sheet-stabilizing metal ions and, as a result, can be arbitrarily shifted in either direction.

The binding of metal ions was monitored by titration of peptide CCM with Cu^{2+} and Zn^{2+} (Fig. 4) for which the $[\Theta]_{208}$ values were recorded by CD spectroscopy. The helical content was decreased by the addition of Cu^{2+} or Zn^{2+} ions, while the β-sheet content continuously increased. These results clearly show that metal complexation triggers the reversion of the TFE-induced helical conformation of peptide CCM to a β-sheet structure. Additionally, the similar behaviour of Cu^{2+} and Zn^{2+} indicates that the conformational change is not influenced by oxidative effects.[18] Although it is not exclusively the His side chain that can be involved in metal ion binding but also backbone NH and CO, the complete loss of the ability of the non-His-containing peptide CC to change its conformation on metal titration reveals the importance of His as a major complexing component.

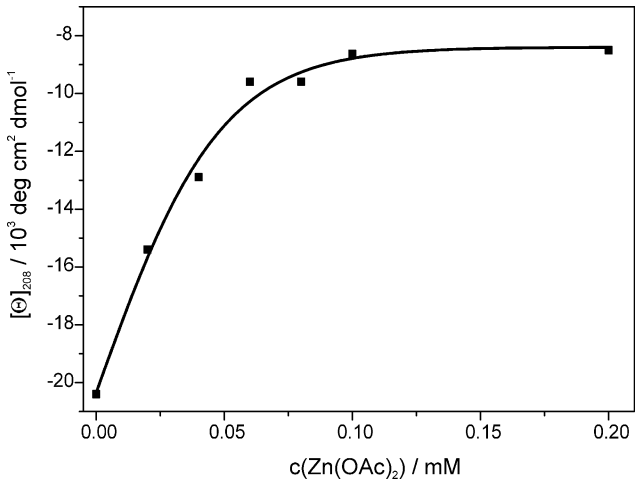

Fig. 4 Zn^{2+} titration profile of 0.1 mM peptide CCM (40% TFE, pH 7.4), as followed by CD spectroscopy at $\lambda = 208$ nm.

With the peptide model introduced here, we have a simple system which now can be used for a systematic study of the impact of different metal ions at their different oxidation states on peptide secondary structures on a molecular level. The design is based on an α-helical coiled coil peptide. Histidine mutations were incorporated into the heptad repeat to generate possible complexation sites for Cu^{2+} and Zn^{2+} ions. Metal ion complexation has been shown to be a strong trigger for a secondary structure switch from an α-helix to a β-sheet. The capture of Cu^{2+} and Zn^{2+} ions by EDTA reversed the β-sheet formation to an α-helical structure. Thus, not only can switching between α-helix and β-sheet be easily controlled in both directions, but even β-sheet formation can be reversed.

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